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SUBTITLE: Determination of the Minimum Effective Pyridostigmine Pretreatment Dose in Monkeys Challenged with 5 X LD_{50}Soman and Treated with Atropine/2-PAM

PRINCIPAL INVESTIGATOR: Carl T. Olson, Ph.D.

CONTRACTING ORGANIZATION: Battelle Memorial Institute
Columbus, Ohio 43201-2693

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Columbus, Ohio 43201-2693

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AMENDED FINAL REPORT

on

TASK 92-30

DETERMINATION OF THE MINIMUM EFFECTIVE PYRIDOSTIGMINE PRETREATMENT DOSE IN MONKEYS CHALLENGED WITH 5 X LD_{50} SOMAN AND TREATED WITH ATROPINE/2-PAM

to

U.S. ARMY MEDICAL RESEARCH AND MATERIEL COMMAND

Carl T Olson 12/22/97

Study Director Date

BATTELLE
Medical Research and Evaluation Facility (West Jefferson, OH)
505 King Avenue, Columbus, OH 43201-2693

SC 920203

AMENDED QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to the Study Director and management as follows:

Phase Inspected	Date Inspected	Date Reported to Study Director	Date of Report to Management
Dilution/Aliquotting of XGD & 2-PAM	2-10-93	3-1-93	3-1-93
NMR Analysis	2-15-93	3-1-93	3-1-93
Analysis/ID Confirmation of Atropine	2-15-93	3-1-93	3-1-93
ID/Purity of Soman	2-15-93	3-1-93	3-1-93
IR Analysis of 2-PAM	2-16-93	3-1-93	3-1-93
HPLC Identity Confirmation	2-16-93	8-31-93	8-31-93
Sedation, Hair Clipping, Body Weights, Skin Marking, Blood Collection	2-22-93	3-1-93	3-1-93
Loading/Weighing of Syringes, IM Dosing, Observations, Post-Dosing Weights	2-23-93	3-1-93	3-1-93
Post-Pyridostigmine Dosing Blood Analysis	3-2-93	3-31-93	3-31-93
Pyridostigmine Dilution, Body Weights, IV Catheterization, Chairing, Pre-Dose & +5 Minute Blood Collection, IM Dosing	3-2-93	3-31-93	3-31-93
Characterization of Pyridostigmine Bromide Standard	3-4-93	3-31-93	3-31-93
Necropsy, Perfusion, Tissue Harvest	3-9-93	3-9-93	3-9-93
Syringe Loading, Dosing, Blood Collection, 2-PAM/Atropine Treatment	4-13-93	5-3-93	5-3-93
Body/Syringe Weights, Femoral Venipuncture, N-G Tube Dosing, IV Catheter Placement, Chairing, 30 & 45 Minute Blood Collection, COBAS Analysis	6-22-93	6-30-93	6-30-93
Dose Calculation, Syringe Loading/Weighing, Oral Dosing, 135 & 145 Minute Blood Collection, COBAS Analysis, Observations	8-10-93	8-31-93	8-31-93
Perfusion, Necropsy	8-13-93	8-31-93	8-31-93

AMENDED QUALITY ASSURANCE STATEMENT SC 920203
(Continued)

Phase Inspected	Date Inspected	Date Reported to Study Director	Date of Report to Management
Pyridostigmine Dose Preparation, Syringe Loading/Weights, Restraint, Blood Collection, Pyridostigmine/GD Dosing, Observations, COBAS Analysis	9-21-93	10-1-93	10-1-93
Hair Clipping, Skin Marking, Blood Collection, GD Aliquotting, Anesthetization	9-23-93	10-1-93	10-1-93
Data/Study File Audits	2-23-93	2-23-93	2-25-93
	6-24-93	6-24-93	7-12-93
	8-12-93	9-20-93	9-23-93
	9-23-93	9-23-93	9-23-93
	11-12-93	11-12-93	11-22-93
	12-7-93	12-7-93	12-13-93
Data/Study File Audits (continued)	12-13-93	12-13-93	12-22-93
	12-30-93	12-30-93	1-12-94
	1-10-94	1-10-94	1-12-94
	2-15-94	2-15-94	3-2-94
	12-2-94	12-2-94	12-15-94
Audit Draft Final Report	12-22-94	12-22-94	1-16-95
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Audit Final Report	8-28-95	8-28-95	8-30-95
Audit Draft Amended Final Report	12-15-97	12-15-97	12-19-97
Audit Amended Final Report	12-22-97	12-22-97	12-22-97

Elisha Morrison 12/22/97

Elisha N. Morrison, M.S. Date
Quality Assurance Specialist

In reviewing the Final Report for MREF Task 92-30, "Determination of the Minimum Effective Pyridostigmine Pretreatment Dose in Monkeys Challenged with 5 x LD_{50} Soman and Treated with Atropine/2-PAM", errors and omissions were noted. This amended report is to correct those oversights. A copy of corrected pages is attached to this amended report.

1. Change:

Delete from Appendix D, "TABLE D-3. EMPIRICALLY OBSERVED AND QUADRATICALLY SMOOTHED VALUES OF CMAX AND TMAX FOR PHASE II EXPERIMENTS" placed after "TABLE D-4. PERCENT AChE INHIBITION IN RESPONSE TO i.m. PYR IN PHASE II EXPERIMENTS", and also delete the following page, "TABLE D-4. DATA LISTING OF PHASE III RESULTS".

Reason for change:

These data are presented in Tables D-5 and D-6, respectively. Tables were added to the Final Report following the recommendations of reviewers of a draft Final Report, and the tables originally numbered D-3 and D-4 were not removed when the Final Report was compiled.

2. Change:

Add footnotes to Tables D-1, D-6, D-9, D-10, D-11, D-12, D-15, D-18, and D-21 to indicate the time of sacrifice for animals that were euthanatized between 48 hr and 10 days. Add the following footnotes to the indicated Tables in Appendix D, Data and Statistical Analyses:

Table D-1:	"Euthanatized because of moribund condition on day 4" for animals 7D4 and 79C.
Table D-6:	"Euthanatized because of moribund condition on day 4" for animals H818, G244, and 78S.
Table D-9:	"Euthanatized because of moribund condition on day 3" for animal 6TR.
Table D-10:	"Euthanatized because of moribund condition on day 3" for animals H398, 73C, 75Z, and 7C6.
	"Euthanatized because of moribund condition on day 4" for animal 6WG.
	"Euthanatized because of moribund condition on day 6" for animals 6TY and 7D6.
Table D-11:	"Euthanatized because of moribund condition on day 3" for animal 6W8.
Table D-12:	"Euthanatized because of moribund condition on day 4" for animals 7D4 and 79C.
Table D-15:	"Euthanatized because of moribund condition on day 4" for animals H818, G244, and 78S.
Table D-18:	"Euthanatized because of moribund condition on day 3" for animal 6TR.
Table D-21:	"Euthanatized because of moribund condition on day 3" for animals H398, 73C, 75Z, 7C6, and 6W8.
	"Euthanatized because of moribund condition on day 4" for animal 6WG.

"Euthanatized because of moribund condition on day 6" for animals 6TY and 7D6.

Reason for change:

These animals were euthanatized prior to 10 days, but after 48 hours, because in my opinion as a veterinarian and the Study Director, the animals were moribund and I believed it inhumane to maintain them on study. Times of euthanasia were noted on the clinical signs forms, but this information was not presented in the Final Report.

3. Change:

In Table D-10, change the calculated GD dose for animal 75Z from "109.4" to "209.4".

Reason for change:

A typographical error in this table was not detected prior to submission of the report.

4. Change:

Add footnotes to Tables D-14, D-17, D-20, and D-23 that "Analysis of time to death based on 48 hr endpoint". In order to have room for this footnote on Table D-14, shorten the footnote under "Analyses of Variance Results Using Censored Values:", "Log-Dose Slope p-value:" from "P-value for the log GD dose slope. P-values for the log-dose slope were nonsignificant for durations within 2 hr and 6hr, so the log GD dose covariate was dropped from the model for duration." to "P-value for the log GD dose slope. Log GD dose covariate was not included in the models for durations."

Reason for change:

Footnotes exist on Tables D-13, D-16, D-19, and D-22 to indicate that the analyses of time to death summarized in these tables were based on 48-hr lethality. Similar footnotes are added to additional tables to prevent misinterpretation.

5. Change:

"TABLE D-14. SUMMARY OF STATISTICAL COMPARISONS BETWEEN CLINICAL SIGNS FOR THE ATR/2-PAM TREATED AND UNTREATED CONTROL GROUP FOR PHASE I EXPERIMENTS", under "Analyses of Variance Results Using Censored Values", change the mean time to onset of convulsions in the "ATR/2-PAM Predicted" column from 2.95 to 2.64 hr, and the S.E. from (4.62) to (4.03). In the same column, change the mean time to onset of miosis from 158 to 136 hr and the S.E. from (354) to (299). In the "Untreated

Predicted" column, change the S.E. for time to onset of convulsions from (1.04) to (1.02). In the same column, change the mean time to onset of miosis from 25.3 to 24.3 hr and the S.E. from (64.3) to (60.5). In the time to onset of convulsions row, change the "Log-Dose Slope p-value" from 0.006 to 0.005.

"TABLE D-23. SUMMARY OF STATISTICAL COMPARISONS BETWEEN CLINICAL SIGNS FOR FOUR TREATMENT GROUPS FROM PHASE V EXPERIMENTS", under "Analysis of Variance Results Using Censored Values", in the "2-PAM Predicted" column, change the mean time to 1st onset of convulsions from 9.75 to NE, delete the S.E. of (10.0), and add a footnote stating "NE - Mean time to onset was not estimable due to the large number of censored values". For the mean time to 1st onset of salivation/bronchial secretions, change the 15.09 to NE and delete the S.E. of (9.70). For the mean time to 1st onset of miosis, change 14.97 to NE and delete the S.E. of (9.44).
In the "2-PAM/DZM Predicted" column, change the mean time to 1st onset of convulsions from 6.81 to NE and delete the S.E. of (6.29). Change the mean time to 1st onset of salivation/bronchial secretions from 5.00 to 27.1 and the S.E. from (2.89) to (23.6). Change the mean time to 1st onset of miosis from 8.73 to 115 and the S.E. from (4.95) to (95.7).
In the "PYR/2-PAM Predicted" column, change the mean time to 1st onset of convulsions 8.78 to NE and delete the S.E. of (8.06). For the mean time to 1st onset of salivation/bronchial secretions, add ")" to the S.E. of "(0.01". Change the mean time to 1st onset of miosis from 0.01 to 0.02.
In the "PYR/2-PAM/DZM Predicted" column, change the mean time to 1st onset of convulsions from 10.21 to NE and delete the S.E. of (9.57). Change the mean time to 1st onset of salivation/bronchial secretions from 0.05 to 0.06 and the S.E. from (0.03) to (0.04).
In the "Chi-Square p-value" column, change the convulsions value from 0.090 to 0.586. Change the salivation/bronchial secretions value from 0.261 to 0.686, and the miosis value from 0.859 to 0.534.
In the "Log-Dose Slope p-value" column, change the 0.011* for convulsions to 0.321.

Page 49 of Final Report of August 1995, 2nd paragraph, conclusions derived from the analyses of the Phase V clinical signs data, item 3), change to read "Log GD dose was significantly related to times of onset of salivation, miosis, and prostration, and time to death, with times to onset predicted to decrease with increasing GD dose". This rewording deletes "convulsions".

Reasons for changes:

Review of the Task 92-30 Final Report, dated August 1995, detected that times to death were not provided in tables of Appendix D for animals that were euthanatized after 48 hr but prior to 10 days. Further investigation by Battelle determined that the times of death of 13 animals that were euthanatized were incorrectly treated in statistical analyses as 10 days. This occurred because times of euthanasia were noted only in the comment fields of the clinical signs forms and were not keyed into the statistical database for clinical signs. This will be corrected in future studies by

having a separate database for euthanasia times.

Battelle conducted an in-depth review of all statistical analyses to assess which results may have been affected by this oversight. The conclusions of that review are:

1) Probit dose-response modeling of lethality data and estimation of LD_{50}s and protective ratios are not affected because these analyses were conducted on the 48-hr lethality data. Animals were not euthanatized prior to 48 hr.

2) The statistical analysis of pyridostigmine-induced AChE-I data was unaffected because blood samples were taken prior to agent exposure.

3) The statistical analysis of time-to-death was unaffected because this analysis treated the time-to-death of animals that did not die before 48 hr as right censored at 48 hr. This step was taken to ensure consistency between the results of the probit dose-response modeling of the 48-hr lethality data and the analysis of the time-to-death data.

4) The statistical analysis of durations of clinical signs were unaffected because this analysis was conducted on duration of signs during the first 2 hr after GD injection and the duration of signs during the first 6 hr after GD injection.

5) The statistical analysis of time to onset of clinical signs was affected because onset times were treated as right censored at either time of death or at 240 hr (10 days) if the sign was not observed. For euthanatized animals, time to onset was treated correctly if the sign was observed prior to euthanasia and was incorrectly treated as right censored at 240 hr if the sign was not observed. The correct response for the latter group of animals is to treat the time to onset as right censored at the time of euthanasia.

For each clinical sign, three types of analyses were conducted on the onset data:

(i) Descriptive statistics of times to onset based on nonmissing uncensored data. Because this analysis included only animals for which the sign was observed, no changes are needed.

(ii) Fisher's exact test of incidence of clinical sign. In this analysis, each animal was given a score of "YES" if the sign was observed and a score of "NO" if the sign was not observed. For animals that did not exhibit a given sign, the score was recorded as "NO" if the animal died prior to 10 days, was euthanatized prior to 10 days, or survived to 10 days. Therefore, the Fisher's exact tests were not affected.

(iii) Parametric analyses of variance (ANOVA) appropriate for censored data. As stated above, this analysis may have been performed incorrectly if the sign was not observed for

one or more of the euthanatized animals.

The time-to-onset data were recomputed for all animals. Time to onset was treated as right censored at time of euthanasia for euthanatized animals that did not exhibit a given sign. The revised data were then compared to the data analyzed for the August 1995 Final Report. Based on this review, the parametric ANOVA analyses were performed on incorrect data in the August 1995 Final Report for the following endpoints: Phase I time to onset of convulsions, Phase I time to onset of miosis, Phase V time to onset of convulsions, Phase V time to onset of salivation, and Phase V time to onset of miosis.

These five analyses were redone using the corrected data. Results were essentially the same for all of the analyses except for the analysis of Phase V time to onset of convulsions. Previous results (page 49 of August 1995 Final Report) indicated that the time to onset of convulsions was significantly related to log GD dose in Phase V. The relationship between time to onset of convulsions and log GD dose is not statistically significant based on the revised analysis.

6. Change:

On page 46 of the August 1995 Final Report, second paragraph, in the conclusions derived from analyses of the Phase I clinical signs data, delete "4) Mean predicted time to death for ATR/2-PAM treated animals was significantly greater than that for untreated animals," and change "5)" to "4)", and "6)" to "5)".

Reason for change:

Although in Table D-14 the p-value for the log GD dose slope for time to death is significant at the $p<0.05$ level, the predicted time to death for ATR/2-PAM treated animals is significantly greater than that for untreated animals only at the $p=0.06$ level.

Battelle personnel believe that revisions do not affect the overall conclusions of the study. The primary endpoint of the study was lethality by 48 hr, and all conclusions based on this endpoint remain the same. I apologize for any problems oversights in the preparation of this report may have caused.

Carl T. Olson, D.V.M., Ph.D.
Study Director

AMENDED TABLE D-1. PHASE I DATA LISTING

Treatment Group	Animal	Date	Target GD Dose (μg/kg)	Calculated [a] GD Dose (μg/kg)	48-Hour Results	Body Weight (kg)	Baseline AChE Activity (U/mL)
Atropine/2-PAM	H489	2/23/93	9.2	9.1	Alive	7.9	6.62
Atropine/2-PAM	H486	2/23/93	15.0	14.9	Alive	7.2	10.21
Atropine/2-PAM	G708	2/25/93	16.0	15.6	Dead	9.0	8.98
Atropine/2-PAM	7D4	3/02/93	17.0	17.0	Alive [b]	8.2	9.78
Atropine/2-PAM	79C	3/09/93	18.0	18.1	Alive [b]	7.7	11.72
Atropine/2-PAM	7BY	3/09/93	20.0	20.3	Alive	8.0	10.28
Atropine/2-PAM	6NL	2/25/93	21.0	21.7	Alive	8.2	10.11
Atropine/2-PAM	G757	3/04/93	23.0	23.1	Dead	8.2	9.98
Atropine/2-PAM	H263	3/02/93	25.0	24.9	Dead	8.0	9.22
Atropine/2-PAM	7BM	3/04/93	27.0	26.8	Dead	8.1	8.43
Untreated	G867	2/25/93	4.5	3.4	Alive	9.2	11.22
Untreated	6TM	2/23/93	5.5	5.2	Dead	8.4	10.68
Untreated	H444	3/02/93	5.3	5.4	Alive	8.2	8.28
Untreated	H413	2/25/93	5.8	5.7	Alive	8.7	9.72
Untreated	77L	2/23/93	8.3	6.9	Dead	6.9	9.10
Untreated	7CG	3/02/93	7.9	7.8	Dead	7.1	10.97

[a] Calculated doses based on weight losses of syringes and chemical analysis of dosing solution.
[b] Euthanatized because of moribund condition on day 4.

AMENDED TABLE D-6. DATA LISTING OF PHASE III RESULTS

Animal	Body Weight (kg)	Date	GD [a] Dose (μg/kg)	PYR Dose (μg/kg)	Baseline AChE Activity (U/mL)	Percent AChE Inhibition	48-Hour Results
75G	6.6	04/20/93	31.9	0.0	10.2	3.7	Died
78J	7.6	04/20/93	32.1	0.0	10.7	2.8	Died
7CA	7.3	05/25/93	30.9	0.0	9.4	2.7	Died
H355	7.9	05/25/93	32.2	0.0	8.7	2.8	Died
6RA	7.2	04/20/93	31.6	4.0	13.2	10.7	Lived
79Y	7.4	04/20/93	32.0	4.0	9.4	6.4	Lived
G654	8.4	04/27/93	32.6	4.0	9.5	5.5	Lived
H831	7.9	04/27/93	32.5	4.0	9.2	6.9	Died
78X	7.6	05/04/93	32.2	4.0	11.7	5.2	Lived
H818	7.4	05/04/93	31.4	4.0	8.0	7.9	Lived [b]
6WB	8.1	05/18/93	33.3	4.0	10.2	3.1	Lived
75F	7.8	05/18/93	32.5	4.0	9.7	5.6	Lived
76P	7.3	05/25/93	32.1	4.0	10.4	9.5	Died
7AH	7.3	06/08/93	32.6	4.0	9.9	8.4	Lived
77K	7.8	04/06/93	31.4	8.4	9.0	13.9	Lived
G244	7.4	04/06/93	32.2	8.4	11.5	6.0	Lived [b]
6Z1	7.9	04/13/93	32.5	8.4	9.5	12.9	Lived
78Y	8.0	04/13/93	32.5	8.4	10.0	14.9	Lived
6PJ	7.3	04/27/93	32.2	8.4	10.1	18.6	Lived
H436	7.7	04/27/93	30.6	8.4	11.8	9.1	Lived
6W2	7.6	05/18/93	32.0	8.4	11.9	13.0	Lived
H602	7.9	05/18/93	32.9	8.4	8.0	10.7	Lived
H432	7.8	05/25/93	31.9	8.4	9.3	12.4	Died
6XR	7.4	06/08/93	31.3	8.4	8.8	9.7	Lived
77V	6.3	04/06/93	32.9	24.0	11.6	28.8	Died
H632	7.5	04/06/93	32.6	24.0	10.8	32.7	Lived
6XC	7.4	04/13/93	33.0	24.0	8.6	23.8	Died
H227	7.4	04/13/93	33.0	24.0	8.5	23.2	Lived
71D	7.6	05/04/93	32.0	24.0	8.9	28.9	Lived
H789	6.9	05/04/93	32.0	24.0	8.4	29.8	Lived
78S	7.4	05/18/93	33.3	24.0	11.3	29.1	Lived [b]
H282	8.1	05/18/93	32.6	24.0	8.9	33.4	Lived
H816	7.2	05/25/93	32.7	24.0	7.4	30.1	Died
H483	7.5	06/08/93	31.8	24.0	9.0	27.6	Lived

[a] Targeted GD dose was 32.5 μg/kg. GD doses in this column are based on weight losses of syringes and initial chemical concentration analysis of the dosing solution.

[b] Euthanatized because of moribund condition on day 4.

AMENDED TABLE D-9. AChE INHIBITION AND LETHALITY RESULTS FOR PHASE IV EXPERIMENTS: 40 μg/kg PYR i.g.; 5 X 48-hr GD LD50 150 MIN FOLLOWING PYR; ATR/2-PAM TREATMENT

Date	Animal	Body Weight (kg)	Baseline AChE Activity[a] -10 min (U/mL)	Baseline AChE Activity[a] -5 min U/mL	AChE-I[b] -15 min (%)	AChE-I[b] -5 min (%)	Calculated[c] PYR Dose (μg/kg)	Calculated[c] GD Dose (μg/kg)	48-Hour Results
8/03/93	75H	6.1	10.1	9.8	-1.6	2.1	40	32.9	Alive
8/03/93	H167	6.4	7.9	7.7	0.3	-2.0	40	33.2	Alive
8/09/93	6TR	7.3	10.0	10.0	2.8	-1.8	40	32.2	Alive [d]
8/09/93	5U3	7.3	10.0	10.1	9.4	12.4	40	32.1	Dead
8/10/93	H843	7.3	10.1	9.9	12.5	9.4	40	31.9	Alive
8/10/93	G923	7.3	11.3	11.2	4.2	3.1	40	31.8	Alive
8/23/93	H237	6.9	11.4	11.2	2.0	2.2	40	31.5	Alive
8/23/93	7CU	7.2	8.9	9.0	-3.0	-2.3	39	31.2	Alive
8/24/93	7BK	7.3	9.9	10.3	15.7	14.2	39	32.1	Dead
8/24/93	6XM	7.2	13.1	13.1	-5.6	-2.3	40	32.3	Alive

[a] AChE activity measured in blood samples drawn approximately 10 and 5 min prior to the intragastric dosing of PYR.
[b] AChE-I values of blood taken approximately 15 and 5 min prior to injection of GD (approximately 135 and 145 min after i.g. dosing of PYR). AChE-I calculations based on -5 min baseline AChE activity value.
[c] Doses calculated from weight losses of syringes and concentrations of dosing solutions based on chemical analyses.
[d] Euthanatized because of moribund condition on day 3.

AMENDED TABLE D-10. PHASE V DATA LISTING

Treatment Group	Date	Animal	Target GD Dose (μg/kg)	Calculated [a] GD Dose (μg/kg)	Body Weight (kg)	Percent AChE Inhibition	48-Hour Results	10-Day Results
ATR/2-PAM/DZM	10/19/93	7CK	7.3	5.8	6.6	-2.3	Alive	Alive
ATR/2-PAM/DZM	10/12/93	71G	7.5	6.6	7.9	-1.3	Alive	Alive
ATR/2-PAM/DZM	09/21/93	78V	10.0	7.3	5.1	-0.2	Alive	Alive
ATR/2-PAM/DZM	09/28/93	6S4	10.5	10.9	8.2	3.2	Died	Died
ATR/2-PAM/DZM	09/24/93	75U	11.0	11.6	8.3	-0.1	Died	Died
ATR/2-PAM/DZM	09/14/93	7AU	12.0	12.3	7.7	1.0	Died	Died
ATR/2-PAM/DZM	09/10/93	6W6	15.0	15.3	6.9	1.6	Died	Died
ATR/2-PAM/DZM	09/03/93	7CC	18.0	17.9	6.5	-1.3	Alive	Died
ATR/2-PAM/DZM	09/07/93	H453	20.5	19.4	7.1	-2.2	Died	Died
ATR/2-PAM/DZM	08/31/93	H264	25.0	28.5	7.4	4.9	Died	Died
ATR/2-PAM	10/12/93	H482	5.5	5.7	8.4	-6.6	Alive	Alive
ATR/2-PAM	10/12/93	66P	7.0	6.9	7.3	0.4	Alive	Alive
ATR/2-PAM	10/19/93	75P	8.5	8.5	8.0	-2.3	Alive	Alive
ATR/2-PAM	09/28/93	7AC	8.0	7.9	8.0	0.8	Died	Died
ATR/2-PAM	09/24/93	74A	8.0	8.5	6.9	-2.8	Alive	Alive
ATR/2-PAM	09/21/93	6VZ	10.0	10.0	6.9	1.3	Died	Died
ATR/2-PAM	10/19/93	H258	13.0	13.2	7.0	-1.1	Died	Died
ATR/2-PAM	09/28/93	6RB	14.0	13.3	5.7	3.0	Died	Died

AMENDED TABLE D-10.
(Continued)

Treatment Group	Date	Animal	Target GD Dose (μg/kg)	Calculated [a] GD Dose (μg/kg)	Body Weight (kg)	Percent AChE Inhibition	48-Hour Results	10-Day Results
PYR/ATR/2-PAM	08/31/93	H398	80.0	79.4	5.8	2.2	Alive	Died [b]
PYR/ATR/2-PAM	09/14/93	6WG	130.0	129.4	6.0	9.8	Alive	Died [c]
PYR/ATR/2-PAM	09/03/93	6TY	160.0	158.3	6.9	7.8	Alive	Died [d]
PYR/ATR/2-PAM	09/21/93	73C	175.0	173.8	7.0	7.0	Alive	Died [b]
PYR/ATR/2-PAM	10/12/93	75Z	210.0	209.4	8.0	8.6	Alive	Died [b]
PYR/ATR/2-PAM	10/19/93	H525	215.0	212.3	7.4	9.5	Died	Died
PYR/ATR/2-PAM	09/10/93	6T4	200.0	199.7	7.5	7.9	Died	Died
PYR/ATR/2-PAM	09/28/93	73P	210.0	208.3	6.6	9.5	Died	Died
PYR/ATR/2-PAM	09/24/93	H074	210.0	208.4	8.0	7.5	Died	Died
PYR/ATR/2-PAM	09/07/93	H585	260.0	257.8	7.0	3.3	Died	Died
PYR/ATR/2-PAM/DZM	10/19/93	79P	60.0	60.3	7.8	5.4	Alive	Alive
PYR/ATR/2-PAM/DZM	10/12/93	H472	75.0	76.1	7.7	1.4	Died	Died
PYR/ATR/2-PAM/DZM	09/28/93	7C6	75.0	75.4	7.3	1.3	Alive	Died [b]
PYR/ATR/2-PAM/DZM	08/31/93	5R2	80.0	78.1	5.3	4.5	Alive	Died
PYR/ATR/2-PAM/DZM	09/24/93	7C4	95.0	96.4	7.9	5.7	Died	Died
PYR/ATR/2-PAM/DZM	09/21/93	H309	110.0	108.8	7.7	8.4	Died	Died
PYR/ATR/2-PAM/DZM	09/14/93	H300	130.0	127.9	7.0	6.6	Died	Died
PYR/ATR/2-PAM/DZM	09/03/93	7D6	160.0	158.6	7.5	4.7	Alive	Died [d]
PYR/ATR/2-PAM/DZM	09/10/93	H612	200.0	198.7	7.5	5.7	Died	Died
PYR/ATR/2-PAM/DZM	09/07/93	7AL	260.0	258.9	6.9	6.7	Died	Died

[a] GD doses calculated using weight losses of syringes and chemical analysis of dosing solution.
[b] Euthanatized because of moribund condition on day 3.
[c] Euthanatized because of moribund condition on day 4.
[d] Euthanatized because of moribund condition on day 6.

AMENDED TABLE D-11. DATA LISTING FOR ADDITIONAL GROUP OF ANIMALS INJECTED WHILE RESTRAINED IN CAGES AND TREATED WITH ATR/2-PAM

Treatment Group	Date	Animal	Target GD Dose (μg/kg)	Calculated [a] GD Dose (μg/kg)	Body Weight (kg)	Baseline AChE Activity (U/mL)	48-Hour Results	10-Day Results
ATR/2-PAM (Cage)	10/26/93	5WT	20.5	20.1	7.7	8.5	Died	Died
ATR-2-PAM (Cage)	10/26/93	I438	20.5	20.6	7.8	9.7	Died	Died
ATR-2-PAM (Cage)	10/26/93	74H	20.5	20.6	7.3	11.9	Died	Died
ATR-2-PAM (Cage)	10/26/93	7C9	20.5	20.6	6.2	8.7	Died	Died
ATR-2-PAM (Cage)	10/26/93	6W8	20.5	20.7	7.8	10.8	Alive	Died [b]

[a] GD doses calculated using weight losses of syringes and chemical analysis of dosing solution.
[b] Euthanatized because of moribund condition on day 3.

AMENDED TABLE D-12. DATA LISTING OF CLINICAL SIGN ENDPOINTS FOR PHASE I

Treatment Group	Calculated GD Dose[a] (μg/kg)	Animal	Clinical Sign	Time to onset (hr)	Time to last obs. (hr)	Duration of sign in 1st 2 hr (hr)	Duration of sign in 1st 6 hr (hr)
ATR/2-PAM	9.1	H489	Appears Normal	18.00	240.00	0.00	0.00
			Tremors	0.00	6.00	1.25	5.25
			Convulsions	-[b]	-	0.00	0.00
			Salivation[c]	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	12.00	2.00	6.00
			Prostration	0.23	1.00	0.77	0.77
			Death	-	-	-	-
ATR/2-PAM	14.9	H486	Appears Normal	120.00	240.00	0.00	0.00
			Tremors	0.00	96.00	2.00	2.00
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	12.00	0.50	3.50
			Miosis	-	-	0.00	0.00
			Mydriasis	0.25	12.00	1.75	5.75
			Prostration	0.30	48.00	1.70	3.70
			Death	-	-	-	-
ATR/2-PAM	15.6	G708	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	1.25	1.25	1.25
			Convulsions	0.38	1.25	0.87	0.87
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	1.28	1.28	1.28
			Prostration	0.15	1.28	1.13	1.13
			Death	1.28	-	-	-

AMENDED TABLE D-12
(Continued)

Treatment Group	Calculated GD Dose[a] (μg/kg)	Animal	Clinical Sign	Time to onset (hr)	Time to last obs. (hr)	Duration of sign in 1st 2 hr (hr)	Duration of sign in 1st 6 hr (hr)
ATR/2-PAM	17.0	7D4	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	96.00	1.75	5.75
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	96.00	2.00	6.00
			Miosis	4.00	12.00	0.00	2.00
			Mydriasis	0.00	4.00	2.00	4.00
			Prostration	0.03	96.00	1.97	5.97
			Death [d]	-	-	-	-
ATR/2-PAM	18.1	79C	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	72.00	2.00	4.00
			Convulsions	0.10	0.25	0.15	0.15
			Salivation	0.25	96.00	1.50	5.50
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	12.00	2.00	6.00
			Prostration	0.08	12.00	1.92	5.92
			Death [d]	-	-	-	-
ATR/2-PAM	20.3	7BY	Appears Normal	192.00	240.00	0.00	0.00
			Tremors	0.00	192.00	2.00	6.00
			Convulsions	-	-	0.00	0.00
			Salivation	1.75	6.00	0.25	4.25
			Miosis	-	-	0.00	0.00
			Mydriasis	0.25	12.00	1.75	5.75
			Prostration	0.37	48.00	1.63	5.63
			Death	-	-	-	-

AMENDED TABLE D-12
(Continued)

Treatment Group	Calculated GD Dose[a] (μg/kg)	Animal	Clinical Sign	Time to onset (hr)	Time to last obs. (hr)	Duration of sign in 1st 2 hr (hr)	Duration of sign in 1st 6 hr (hr)
ATR/2-PAM	21.7	6NL	Appears Normal	48.00	72.00	0.00	0.00
			Tremors	0.00	72.00	1.75	1.75
			Convulsions	0.05	0.25	0.20	0.20
			Salivation	0.25	101.67	1.75	5.75
			Miosis	72.00	101.67	0.00	0.00
			Mydriasis	0.00	6.00	2.00	6.00
			Prostration	0.07	101.67	1.93	5.93
			Death	101.67	-	-	-
ATR/2-PAM	23.1	G757	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	1.15	0.90	0.90
			Convulsions	0.05	0.25	0.20	0.20
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	1.15	1.15	1.15
			Prostration	0.07	1.15	1.08	1.08
			Death	1.15	-	-	-
ATR/2-PAM	24.9	H263	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	0.25	0.25	0.25
			Convulsions	-	-	0.00	0.00
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	0.25	0.25	0.25
			Prostration	0.07	0.37	0.30	0.30
			Death	0.37	-	-	-

AMENDED TABLE D-12
(Continued)

Treatment Group	Calculated GD Dose[a] (μg/kg)	Animal	Clinical Sign	Time to onset (hr)	Time to last obs. (hr)	Duration of sign in 1st 2 hr (hr)	Duration of sign in 1st 6 hr (hr)
ATR/2-PAM	26.8	7BM	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	12.00	2.00	6.00
			Convulsions	0.02	3.00	0.23	1.23
			Salivation	0.00	12.00	1.25	5.25
			Miosis	0.00	12.00	0.50	3.50
			Mydriasis	0.50	3.00	1.50	2.50
			Prostration	0.03	12.00	1.97	5.97
			Death	20.43	-	-	-
Untreated	3.4	G867	Appears Normal	0.00	240.00	2.00	6.00
			Tremors	-	-	0.00	0.00
			Convulsions	-	-	0.00	0.00
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	-	-	0.00	0.00
			Prostration	-	-	0.00	0.00
			Death	-	-	-	-
Untreated	5.2	6TM	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	12.00	1.25	5.25
			Convulsions	0.27	4.00	0.98	1.98
			Salivation	0.00	30.10	2.00	6.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	30.10	2.00	6.00
			Prostration	0.25	30.10	1.75	5.75
			Death	30.10	-	-	-

AMENDED TABLE D-12
(Continued)

Treatment Group	Calculated GD Dose[a] (μg/kg)	Animal	Clinical Sign	Time to onset (hr)	Time to last obs. (hr)	Duration of sign in 1st 2 hr (hr)	Duration of sign in 1st 6 hr (hr)
Untreated	5.4	H444	Appears Normal	4.00	240.00	0.00	2.00
			Tremors	0.00	1.00	1.00	1.00
			Convulsions	-	-	0.00	0.00
			Salivation	-	-	0.00	0.00
			Miosis	0.25	4.00	1.75	3.75
			Mydriasis	-	-	0.00	0.00
			Prostration	-	-	0.00	0.00
			Death	-	-	-	-
Untreated	5.7	H413	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	48.00	2.00	6.00
			Convulsions	0.18	6.00	1.07	5.07
			Salivation	0.00	48.00	2.00	6.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	6.00	0.75	4.75
			Prostration	0.23	92.65	1.77	5.77
			Death	92.65	-	-	-
Untreated	6.9	77L	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	6.00	2.00	6.00
			Convulsions	0.12	3.00	1.63	2.63
			Salivation	0.00	12.00	2.00	6.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	12.00	2.00	6.00
			Prostration	0.13	12.00	1.87	5.87
			Death	20.35	-	-	-

AMENDED TABLE D-12
(Continued)

Treatment Group	Calculated GD Dose[a] (μg/kg)	Animal	Clinical Sign	Time to onset (hr)	Time to last obs. (hr)	Duration of sign in 1st 2 hr (hr)	Duration of sign in 1st 6 hr (hr)
Untreated	7.8	7CG	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	0.75	0.75	0.75
			Convulsions	0.08	0.25	0.17	0.17
			Salivation	0.00	0.93	0.93	0.93
			Miosis	0.25	0.50	0.25	0.25
			Mydriasis	0.00	0.75	0.50	0.50
			Prostration	0.10	0.93	0.83	0.83
			Death	0.93	-	-	-

[a] GD doses calculated from weight losses of syringes and chemical analysis of dosing solution.
[b] Sign was not noted during duration of the experiment.
[c] Excessive secretion of saliva or bronchial fluids as evidenced by discharge from the mouth or nares.
[d] Euthanatized in moribund condition on day 4.

AMENDED TABLE D-14. SUMMARY OF STATISTICAL COMPARISONS BETWEEN CLINICAL SIGNS FOR THE ATR/2-PAM TREATED AND UNTREATED CONTROL GROUP FOR PHASE I EXPERIMENTS

Clinical Sign	Incidence of Clinical Sign		Fisher's Exact Test	Endpoint	Wilcoxon Test p-value	Analyses of Variance Results Using Censored Values					
						ATR/2-PAM Predicted		Untreated Predicted		Chi-Square p-value	Log-Dose Slope p-value
	ATR/2-PAM	Untreated				Mean (hr)	(S.E.)	Mean (hr)	(S.E.)		
Tremors	10/10	5/6	0.375	Time to onset	-	0.01	(0.01)	0.03	(0.03)	0.231	0.035 [a]
				Duration, 1st 2 hrs	0.371	1.60	(0.20)	1.17	(0.25)	0.176	-
				Duration, 1st 6 hrs	0.785	3.64	(0.76)	3.17	(0.95)	0.697	-
Convulsions	5/10	4/6	0.633	Time to onset	-	2.64	(4.03)	0.54	(1.02)	0.007 [a]	0.005 [a]
				Duration, 1st 2 hrs	0.213	0.17	(0.14)	0.64	(0.18)	0.032 [a]	-
				Duration, 1st 6 hrs	0.213	0.27	(0.37)	1.64	(0.48)	0.023 [a]	-
Salivation/ Bronchial Secretions	6/10	4/6	1.000	Time to onset	-	0.43	(0.68)	0.13	(0.26)	0.027 [a]	0.022 [a]
				Duration, 1st 2 hrs	0.340	0.73	(0.27)	1.29	(0.36)	0.204	-
				Duration, 1st 6 hrs	0.466	3.03	(0.84)	3.74	(1.16)	0.616	-
Miosis	3/10	2/6	1.000	Time to onset	-	136	(299)	24.3	(60.5)	0.044 [a]	0.041 [a]
				Duration, 1st 2 hrs	0.265	0.05	(0.13)	0.33	(0.17)	0.180	-
				Duration, 1st 6 hrs	0.569	0.55	(0.39)	0.67	(0.51)	0.856	-
Mydriasis	10/10	4/6	0.125	Time to onset	-	0.02	(0.02)	0.26	(0.40)	0.671	0.216
				Duration, 1st 2 hrs	0.181	1.72	(0.23)	0.88	(0.28)	0.019 [a]	-
				Duration, 1st 6 hrs	0.440	4.62	(0.80)	2.88	(0.95)	0.159	-
Prostration	10/10	4/6	0.125	Time to onset	-	0.06	(0.04)	1.25	(1.16)	0.375	0.016 [a]
				Duration, 1st 2 hrs	0.277	1.75	(0.23)	1.14	(0.27)	0.080	-
				Duration, 1st 6 hrs	0.277	4.92	(0.82)	3.57	(0.97)	0.290	-
Death [b]	4/10	3/6	1.000	Time to death	-	33.4	(36.9)	27.6	(36.7)	0.061	0.041 [a]

Explanation of column headings

Incidence of Clinical Sign: Proportion of animals in each group that exhibited the clinical sign.

Fisher's Exact Test: P-value for comparing the incidence of a clinical sign between the ATR/2-PAM group and untreated control group.

Wilcoxon Test: P-value for Wilcoxon's rank sum test comparing nonmissing, uncensored durations between the ATR/2-PAM and untreated control groups. This test was not performed on times to onset since they were determined to be dose-dependent.

Analyses of Variance Results Using Censored Values:

Predicted Mean: The mean value of endpoint predicted by the ANOVA for each group. S.E. is the standard error of the predicted mean. Predicted means were computed at the GD 48 hr LD_{50} for each group, 20.5 μg/kg and 6.50 μg/kg for the ATR/2-PAM group and untreated group, respectively.

Chi-square p-value: P-value comparing the mean predicted values between the ATR/2-PAM and untreated control groups.

Log-Dose Slope p-value: P-value for the log GD dose slope. Log GD dose covariate was not included in the models for durations.

[a] Statistically significant at the 0.05 level.

[b] Analysis of time to death based on 48 hr endpoint.

AMENDED TABLE D-15. DATA LISTING OF CLINICAL SIGN ENDPOINTS FOR PHASE III

PYR Dose (μg/kg i.m.)	Animal	Date	Clinical Sign	Time to onset (hr)	Time to last obs. (hr)	Duration of sign in 1st 2 hr (hr)	Duration of sign in 1st 6 hr (hr)
0.0	75G	04/20/93	Appears Normal	-[a]	-	0.00	0.00
			Tremors	0.00	0.75	0.75	0.75
			Convulsions	0.45	0.92	0.47	0.47
			Salivation[b]	0.00	0.92	0.92	0.92
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	0.92	0.92	0.92
			Prostration	0.03	0.92	0.88	0.88
			Death	0.92	-	-	-
0.0	78J	04/20/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	0.25	0.25	0.25
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	2.42	2.00	2.42
			Miosis	0.00	0.50	0.50	0.50
			Mydriasis	0.00	0.25	0.25	0.25
			Prostration	0.05	2.42	1.95	2.37
			Death	2.42	-	-	-
0.0	7CA	05/25/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	23.97	2.00	6.00
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	23.97	0.75	4.75
			Miosis	0.25	23.97	1.75	5.75
			Mydriasis	0.00	0.50	0.50	0.50
			Prostration	0.03	23.97	1.97	5.97
			Death	23.97	-	-	-
0.0	H355	05/25/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	0.50	0.50	0.50
			Convulsions	0.12	0.50	0.38	0.38
			Salivation	0.00	0.63	0.63	0.63
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	0.63	0.63	0.63
			Prostration	0.05	0.50	0.45	0.45
			Death	0.63	-	-	-
4.0	6RA	04/20/93	Appears Normal	144.00	240.00	0.00	0.00
			Tremors	0.00	96.00	2.00	5.50
			Convulsions	0.18	0.25	0.07	0.07
			Salivation	0.50	0.75	0.25	0.25
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	12.00	2.00	6.00
			Prostration	0.08	12.00	1.92	5.42
			Death	-	-	-	-
4.0	79Y	04/20/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	96.00	1.50	5.50
			Convulsions	-	-	0.00	0.00
			Salivation	0.25	72.00	1.00	1.00
			Miosis	18.00	240.00	0.00	0.00
			Mydriasis	0.00	0.50	0.50	0.50
			Prostration	0.03	6.00	1.97	4.47
			Death	-	-	-	-

AMENDED TABLE D-15.
(Continued)

PYR Dose (μg/kg i.m.)	Animal	Date	Clinical Sign	Time to onset (hr)	Time to last obs. (hr)	Duration of sign in 1st 2 hr (hr)	Duration of sign in 1st 6 hr (hr)
4.0	G654	04/27/93	Appears Normal	48.00	240.00	0.00	0.00
			Tremors	0.00	3.00	1.25	2.25
			Convulsions	0.05	0.25	0.20	0.20
			Salivation	3.00	12.00	0.00	3.00
			Miosis	24.00	48.00	0.00	0.00
			Mydriasis	0.00	4.50	2.00	4.50
			Prostration	0.05	12.00	1.95	5.95
			Death	-	-	-	-
4.0	H831	04/27/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	0.82	0.82	0.82
			Convulsions	0.10	0.82	0.72	0.72
			Salivation	0.00	0.50	0.50	0.50
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	0.82	0.82	0.82
			Prostration	0.05	0.82	0.77	0.77
			Death	0.82	-	-	-
4.0	78X	05/04/93	Appears Normal	120.00	144.00	0.00	0.00
			Tremors	0.00	1.25	1.25	1.25
			Convulsions	0.13	1.25	1.12	1.12
			Salivation	0.00	48.00	2.00	6.00
			Miosis	0.75	240.00	0.75	4.75
			Mydriasis	0.00	0.75	0.75	0.75
			Prostration	0.05	12.00	1.95	5.95
			Death	-	-	-	-
4.0	H818	05/04/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	72.00	2.00	6.00
			Convulsions	0.63	4.00	0.62	1.62
			Salivation	0.50	96.00	1.50	5.50
			Miosis	24.00	72.00	0.00	0.00
			Mydriasis	0.00	6.00	2.00	6.00
			Prostration	0.05	96.00	1.95	5.95
			Death [c]	-	-	-	-
4.0	6WB	05/18/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	192.00	1.75	5.25
			Convulsions	4.10	4.50	0.00	0.40
			Salivation	0.00	2.00	0.50	0.50
			Miosis	2.50	216.00	0.00	3.50
			Mydriasis	0.00	1.00	1.00	1.00
			Prostration	0.03	12.00	1.97	4.97
			Death	-	-	-	-
4.0	75F	05/18/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	216.00	1.00	4.50
			Convulsions	0.15	1.50	1.35	1.35
			Salivation	0.00	48.00	1.50	5.00
			Miosis	120.00	216.00	0.00	0.00
			Mydriasis	0.00	3.50	1.75	3.25
			Prostration	0.03	12.00	1.97	5.97
			Death	-	-	-	

AMENDED TABLE D-15.
(Continued)

PYR Dose (μg/kg i.m.)	Animal	Date	Clinical Sign	Time to onset (hr)	Time to last obs. (hr)	Duration of sign in 1st 2 hr (hr)	Duration of sign in 1st 6 hr (hr)
4.0	76P	05/25/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	1.35	1.35	1.35
			Convulsions	0.40	1.25	0.60	0.60
			Salivation	0.25	1.25	1.00	1.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	1.35	1.35	1.35
			Prostration	0.05	1.35	1.30	1.30
			Death	1.35	-	-	-
4.0	7AH	06/08/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	1.50	1.50	1.50
			Convulsions	0.12	1.25	0.88	0.88
			Salivation	0.75	54.83	0.75	4.75
			Miosis	48.00	54.83	0.00	0.00
			Mydriasis	0.00	48.00	2.00	6.00
			Prostration	0.13	54.83	1.87	5.87
			Death	54.83	-	-	-
8.4	77K	04/06/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	1.25	1.25	1.25
			Convulsions	0.05	0.25	0.20	0.20
			Salivation	0.00	12.00	2.00	6.00
			Miosis	18.00	216.00	0.00	0.00
			Mydriasis	0.00	1.00	1.00	1.00
			Prostration	0.07	12.00	1.93	5.93
			Death	-	-	-	-
8.4	G244	04/06/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	72.00	1.50	5.50
			Convulsions	0.03	12.00	0.22	1.72
			Salivation	0.25	96.00	1.25	3.75
			Miosis	18.00	24.00	0.00	0.00
			Mydriasis	0.00	12.00	2.00	4.50
			Prostration	0.08	96.00	1.92	5.92
			Death [c]	-	-	-	-
8.4	6Z1	04/13/93	Appears Normal	144.00	240.00	0.00	0.00
			Tremors	0.00	5.50	2.00	4.50
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	1.50	1.50	1.50
			Miosis	0.00	72.00	2.00	6.00
			Mydriasis	0.00	0.25	0.25	0.25
			Prostration	0.05	1.00	0.95	0.95
			Death	-	-	-	-
8.4	78Y	04/13/93	Appears Normal	144.00	240.00	0.00	0.00
			Tremors	0.00	3.50	1.75	3.25
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	72.00	1.25	1.25
			Miosis	1.25	72.00	0.75	4.75
			Mydriasis	0.00	0.25	0.25	0.25
			Prostration	0.03	1.25	1.22	1.22
			Death	-	-	-	-

AMENDED TABLE D-15.
(Continued)

PYR Dose (μg/kg i.m.)	Animal	Date	Clinical Sign	Time to onset (hr)	Time to last obs. (hr)	Duration of sign in 1st 2 hr (hr)	Duration of sign in 1st 6 hr (hr)
8.4	6PJ	04/27/93	Appears Normal	48.00	240.00	0.00	0.00
			Tremors	0.00	12.00	2.00	6.00
			Convulsions	0.07	1.00	0.93	0.93
			Salivation	0.25	12.00	1.00	5.00
			Miosis	1.00	48.00	0.50	1.00
			Mydriasis	0.00	1.00	1.00	1.00
			Prostration	0.03	12.00	1.97	5.97
			Death	-	-	-	-
8.4	H436	04/27/93	Appears Normal	96.00	240.00	0.00	0.00
			Tremors	0.00	12.00	1.75	5.75
			Convulsions	-	-	0.00	0.00
			Salivation	-	-	0.00	0.00
			Miosis	5.00	72.00	0.00	1.00
			Mydriasis	0.00	1.25	1.25	1.25
			Prostration	0.05	48.00	1.20	1.20
			Death	-	-	-	-
8.4	6W2	05/18/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	240.00	1.25	4.75
			Convulsions	5.50	12.00	0.00	0.50
			Salivation	0.00	48.00	0.75	0.75
			Miosis	3.00	216.00	0.00	3.00
			Mydriasis	0.00	0.25	0.25	0.25
			Prostration	0.05	2.00	1.95	1.95
			Death	-	-	-	-
8.4	H602	05/18/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	144.00	0.25	3.25
			Convulsions	-	-	0.00	0.00
			Salivation	0.25	0.50	0.25	0.25
			Miosis	18.00	240.00	0.00	0.00
			Mydriasis	0.00	4.50	2.00	4.50
			Prostration	0.03	12.00	1.97	5.97
			Death	-	-	-	-
8.4	H432	05/25/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	0.87	0.87	0.87
			Convulsions	0.23	0.50	0.27	0.27
			Salivation	0.00	0.75	0.75	0.75
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	0.87	0.87	0.87
			Prostration	0.05	0.75	0.70	0.70
			Death	0.87	-	-	-
8.4	6XR	06/08/93	Appears Normal	96.00	240.00	0.00	0.00
			Tremors	0.00	5.50	1.50	4.00
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	1.50	1.00	1.00
			Miosis	1.75	96.00	0.25	4.25
			Mydriasis	0.00	1.25	1.25	1.25
			Prostration	0.05	3.00	1.45	1.95
			Death	-	-	-	-

AMENDED TABLE D-15.
(Continued)

PYR Dose (μg/kg i.m.)	Animal	Date	Clinical Sign	Time to onset (hr)	Time to last obs. (hr)	Duration of sign in 1st 2 hr (hr)	Duration of sign in 1st 6 hr (hr)
24.0	77V	04/06/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	4.00	2.00	4.00
			Convulsions	0.88	1.00	0.12	0.12
			Salivation	0.25	45.58	1.75	5.75
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	4.00	2.00	4.00
			Prostration	0.05	45.58	1.95	5.95
			Death	45.58	-	-	-
24.0	H632	04/06/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	2.50	2.00	2.50
			Convulsions	0.02	2.50	0.73	1.23
			Salivation	1.25	12.00	0.75	4.75
			Miosis	18.00	141.67	0.00	0.00
			Mydriasis	0.00	2.50	2.00	2.50
			Prostration	0.07	12.00	1.93	5.93
			Death	141.67	-	-	-
24.0	6XC	04/13/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	5.50	1.75	4.25
			Convulsions	0.12	5.00	0.88	1.38
			Salivation	0.00	43.33	2.00	6.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	43.33	2.00	6.00
			Prostration	0.02	43.33	1.98	5.98
			Death	43.33	-	-	-
24.0	H227	04/13/93	Appears Normal	24.00	240.00	0.00	0.00
			Tremors	0.00	6.00	2.00	4.50
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	0.75	0.75	0.75
			Miosis	1.75	5.00	0.25	3.25
			Mydriasis	0.00	1.00	1.00	1.00
			Prostration	0.03	1.00	0.97	0.97
			Death	-	-	-	-
24.0	71D	05/04/93	Appears Normal	120.00	144.00	0.00	0.00
			Tremors	0.00	12.00	2.00	5.50
			Convulsions	0.08	0.25	0.17	0.17
			Salivation	-	-	0.00	0.00
			Miosis	2.00	240.00	0.00	2.50
			Mydriasis	0.00	0.50	0.50	0.50
			Prostration	0.07	1.25	1.18	1.18
			Death	-	-	-	-
24.0	H789	05/04/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	68.25	1.75	3.25
			Convulsions	0.15	1.75	1.60	1.60
			Salivation	0.00	68.25	1.50	2.00
			Miosis	5.00	68.25	0.00	1.00
			Mydriasis	0.00	68.25	1.25	1.75
			Prostration	0.03	68.25	1.97	5.97
			Death	68.25	-	-	-

AMENDED TABLE D-15.
(Continued)

PYR Dose (μg/kg i.m.)	Animal	Date	Clinical Sign	Time to onset (hr)	Time to last obs. (hr)	Duration of sign in 1st 2 hr (hr)	Duration of sign in 1st 6 hr (hr)
24.0	78S	05/18/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	48.00	2.00	6.00
			Convulsions	0.07	2.50	1.93	2.43
			Salivation	0.75	48.00	1.25	5.25
			Miosis	0.75	96.00	0.25	0.25
			Mydriasis	0.00	5.00	1.50	4.50
			Prostration	0.03	96.00	1.97	5.97
			Death [c]	-	-	-	-
24.0	H282	05/18/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	72.00	1.25	4.75
			Convulsions	0.05	0.25	0.20	0.20
			Salivation	-	-	0.00	0.00
			Miosis	0.75	240.00	1.25	5.25
			Mydriasis	0.00	0.50	0.50	0.50
			Prostration	0.05	1.25	1.20	1.20
			Death	-	-	-	-
24.0	H816	05/25/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	0.88	0.88	0.88
			Convulsions	-	-	0.00	0.00
			Salivation	0.25	0.88	0.63	0.63
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	0.88	0.88	0.88
			Prostration	0.05	0.88	0.83	0.83
			Death	0.88	-	-	-
24.0	H483	06/08/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	168.00	0.50	3.50
			Convulsions	0.05	0.25	0.20	0.20
			Salivation	0.00	12.00	2.00	6.00
			Miosis	18.00	240.00	0.00	0.00
			Mydriasis	0.00	4.00	2.00	4.00
			Prostration	0.05	3.50	1.95	3.45
			Death	-	-	-	-

[a] Sign was not noted during duration of the experiment.
[b] Excessive secretion of saliva or bronchial fluids.
[c] Euthanatized because of moribund condition on day 4.

AMENDED TABLE D-16. DESCRIPTIVE STATISTICS OF CLINICAL SIGNS FOR THE CONTROL GROUP AND PYR-DOSED GROUPS IN PHASE III EXPERIMENTS BASED ON NONMISSING UNCENSORED ENDPOINTS

Clinical Sign	Endpoint	PYR Dose Group														
		Untreated Control			4 μg/kg			8.4 μg/kg			24 μg/kg			4-24 μg/kg[a]		
		N[b]	Mean (hr)	(S.D.)	N	Mean (hr)	(S.D.)	N	Mean (hr)	(S.D.)	N	Mean (hr)	(S.D.)	N	Mean (hr)	(S.D.)
Tremors	Time to onset	4	0.00	(0.00)	10	0.00	(0.00)	10	0.00	(0.00)	10	0.00	(0.00)	30	0.00	(0.00)
	Duration in 1st 2 hrs	4	0.88	(0.78)	10	1.44	(0.39)	10	1.41	(0.54)	10	1.61	(0.55)	30	1.49	(0.49)
	Duration in 1st 6 hrs	4	1.87	(2.76)	10	3.39	(2.13)	10	3.91	(1.78)	10	3.91	(1.48)	30	3.74	(1.77)
Convulsions	Time to onset	2	0.28	(0.24)	9	0.65	(1.31)	5	1.18	(2.42)	8	0.18	(0.29)	22	0.60	(1.39)
	Duration in 1st 2 hrs	4	0.21	(0.25)	10	0.56	(0.48)	10	0.16	(0.29)	10	0.58	(0.69)	30	0.43	(0.53)
	Duration in 1st 6 hrs	4	0.21	(0.25)	10	0.70	(0.55)	10	0.36	(0.56)	10	0.73	(0.86)	30	0.60	(0.67)
Salivation/ Bronchial Secretions	Time to onset	4	0.00	(0.00)	10	0.53	(0.91)	9	0.08	(0.12)	8	0.31	(0.46)	27	0.31	(0.62)
	Duration in 1st 2 hrs	4	1.07	(0.63)	7	0.90	(0.63)	10	0.97	(0.58)	10	1.06	(0.75)	30	0.98	(0.64)
	Duration in 1st 6 hrs	4	2.18	(1.88)	10	2.75	(2.35)	10	2.02	(2.11)	10	3.11	(2.65)	30	2.63	(2.34)
Miosis	Time to onset	2	0.13	(0.18)	10	33.89	(41.12)	9	7.33	(8.12)	7	6.61	(7.91)	23	15.20	(25.73)
	Duration in 1st 2 hrs	4	0.56	(0.83)	10	0.08	(0.24)	10	0.35	(0.64)	10	0.17	(0.39)	30	0.20	(0.45)
	Duration in 1st 6 hrs	4	1.56	(2.80)	10	0.82	(1.76)	10	2.00	(2.30)	10	1.22	(1.84)	30	1.35	(1.98)
Mydriasis	Time to onset	4	0.00	(0.00)	10	0.00	(0.00)	10	0.00	(0.00)	10	0.00	(0.00)	30	0.00	(0.00)
	Duration in 1st 2 hrs	4	0.57	(0.28)	10	1.42	(0.61)	10	1.01	(0.65)	10	1.36	(0.62)	30	1.26	(0.63)
	Duration in 1st 6 hrs	4	0.57	(0.28)	10	3.02	(2.41)	10	1.51	(1.62)	10	2.56	(1.95)	30	2.36	(2.05)
Prostration	Time to onset	4	0.04	(0.01)	10	0.06	(0.03)	10	0.05	(0.02)	10	0.04	(0.02)	30	0.05	(0.02)
	Duration in 1st 2 hrs	4	1.31	(0.77)	10	1.76	(0.40)	10	1.52	(0.48)	10	1.59	(0.48)	30	1.63	(0.45)
	Duration in 1st 6 hrs	4	2.42	(2.50)	10	4.66	(1.98)	10	3.17	(2.42)	10	3.74	(2.45)	30	3.86	(2.30)
Death	Time to death[c]	4	6.98	(11.35)	2	1.08	(0.38)	1	0.87	(-)	3	29.93	(25.18)	6	15.47	(22.46)

[a] Descriptive statistics for all three PYR dose groups pooled.
[b] For times to onset and death, N is the number of animals that responded. For durations, N is the number of animals in the study groups.
[c] Based on 48-hr endpoint.

AMENDED TABLE D-17. SUMMARY OF STATISTICAL COMPARISONS BETWEEN CLINICAL SIGNS FOR THE CONTROL GROUP AND POOLED PYR-DOSED GROUPS FOR PHASE III EXPERIMENTS

Clinical Sign	Incidence of Clinical Sign		Fisher's Exact Test	Endpoint	Wilcoxon Test p-value	Analyses of Variance Results Using Censored Values				
						Control Group Predicted		4-24 μg/kg Predicted		
	Control	4-24 μg/kg				Mean (hr)	(S.E.)	Mean (hr)	(S.E.)	Chi-Square p-value
Tremors	4/4	30/30	1.000	Time to onset	1.000	0.00	(0.00)	0.00	(0.00)	1.000
				Duration in 1st 2 hrs	0.097	0.88	(0.24)	1.59	(0.09)	0.007 [a]
				Duration in 1st 6 hrs	0.092	1.87	(0.80)	4.18	(0.31)	0.007 [a]
Convulsions	2/4	22/30	0.564	Time to onset	0.463	7.40	(17.71)	1.67	(1.37)	0.554
				Duration in 1st 2 hrs	0.511	0.30	(0.27)	0.44	(0.10)	0.621
				Duration in 1st 6 hrs	0.303	0.33	(0.34)	0.61	(0.12)	0.431
Salivation/ Bronchial Secretions	4/4	27/30	1.000	Time to onset	0.085	0.01	(0.02)	0.16	(0.09)	0.102
				Duration in 1st 2 hrs	0.914	1.52	(0.39)	1.00	(0.12)	0.201
				Duration in 1st 6 hrs	0.810	5.39	(1.86)	2.72	(0.41)	0.161
Miosis	2/4	23/30	0.281	Time to onset	0.030 [a]	0.45	(0.56)	7.93	(3.54)	0.031 [a]
				Duration in 1st 2 hrs	0.273	0.56	(0.24)	0.20	(0.09)	0.160
				Duration in 1st 6 hrs	0.814	1.56	(1.00)	1.35	(0.37)	0.842
Mydriasis	4/4	30/30	1.000	Time to onset	1.000	0.00	(0.00)	0.00	(0.00)	1.000
				Duration in 1st 2 hrs	0.039 [a]	0.84	(0.35)	1.35	(0.12)	0.169
				Duration in 1st 6 hrs	0.041 [a]	1.52	(1.13)	2.68	(0.38)	0.327
Prostration	4/4	30/30	1.000	Time to onset	0.388	0.04	(0.01)	0.05	(0.00)	0.468
				Duration in 1st 2 hrs	0.499	1.48	(0.24)	1.71	(0.08)	0.378
				Duration in 1st 6 hrs	0.321	3.85	(1.32)	4.21	(0.41)	0.789
Death [b]	4/4	6/30	0.005 [a]	Time to death	0.831	2.41	(3.97)	575	(689)	0.007 [a]

Explanation of column headings

Incidence of Clinical Sign: Proportion of animals in each group that exhibited the clinical sign.

4-24 μg/kg: Proportion over all three PYR dose groups (4 μg/kg, 8.4 μg/kg, 24 μg/kg).

Fisher's Exact Test: p-value for comparing the incidence of clinical signs between the untreated control group and the pooled 4-24 μg/kg PYR group.

Wilcoxon Test: p-value for Wilcoxon's rank sum test comparing nonmissing, uncensored durations between the untreated control group and the pooled 4-24 μg/kg PYR group.

Analyses of Variance Using Censored Values:

Predicted Mean: The mean value of endpoint predicted by the ANOVA for each group. S.E. is the standard error of the predicted mean.

Chi-square p-value: p-value for comparing the mean predicted values between the untreated control group and the pooled 4-24 μg/kg PYR group.

[a] Statistically significant at the 0.05 level.

[b] Analysis of time to death based on 48 hr endpoint.

AMENDED TABLE D-18. DATA LISTING OF CLINICAL SIGN ENDPOINTS FOR PHASE IV EXPERIMENTS

Calculated[a] PYR Dose (μg/kg i.g.)	Animal	Date	Clinical Sign	Time to onset (hr)	Time to last obs. (hr)	Duration of sign in 1st 2 hr (hr)	Duration of sign in 1st 6 hr (hr)
40	75H	08/03/93	Appears Normal	-[b]	-	0.00	0.00
			Tremors	0.00	240.00	2.00	6.00
			Convulsions	0.05	4.00	1.45	2.95
			Salivation[c]	0.00	48.00	2.00	6.00
			Miosis	1.00	240.00	0.75	4.75
			Mydriasis	0.00	0.25	0.25	0.25
			Prostration	0.05	12.00	1.95	5.95
			Death	-	-	-	-
40	H167	08/03/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	139.58	2.00	6.00
			Convulsions	0.65	2.50	1.35	1.85
			Salivation	0.00	48.00	2.00	6.00
			Miosis	0.00	72.00	1.25	2.75
			Mydriasis	0.00	0.25	0.25	0.25
			Prostration	0.05	12.00	1.95	5.95
			Death	139.58	-	-	-
40	5U3	08/09/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	0.17	0.17	0.17
			Convulsions	-	-	0.00	0.00
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	0.17	0.17	0.17
			Prostration	0.05	0.17	0.12	0.12
			Death	0.17	-	-	-
40	6TR	08/09/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	4.50	2.00	4.50
			Convulsions	1.17	1.50	0.33	0.33
			Salivation	0.00	72.00	2.00	6.00
			Miosis	3.50	72.00	0.00	2.50
			Mydriasis	0.00	1.75	1.75	1.75
			Prostration	0.03	72.00	1.72	1.72
			Death [d]	-	-	-	-
40	G923	08/10/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	96.00	1.00	1.50
			Convulsions	0.05	0.25	0.20	0.20
			Salivation	0.25	48.00	1.75	5.25
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	12.00	2.00	6.00
			Prostration	0.07	141.00	1.93	5.93
			Death	141.00	-	-	-

AMENDED TABLE D-18
(Continued)

Calculated[a] PYR Dose (μg/kg i.g.)	Animal	Date	Clinical Sign	Time to onset (hr)	Time to last obs. (hr)	Duration of sign in 1st 2 hr (hr)	Duration of sign in 1st 6 hr (hr)
40	H843	08/10/93	Appears Normal	96.00	240.00	0.00	0.00
			Tremors	0.00	12.00	2.00	6.00
			Convulsions	0.57	1.00	0.43	0.43
			Salivation	0.00	1.50	1.50	1.50
			Miosis	18.00	96.00	0.00	0.00
			Mydriasis	0.00	12.00	2.00	5.00
			Prostration	0.05	4.50	1.70	3.20
			Death	-	-	-	-
39	7CU	08/23/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	4.50	2.00	4.00
			Convulsions	-	-	0.00	0.00
			Salivation	0.50	96.00	0.50	0.50
			Miosis	18.00	96.00	0.00	0.00
			Mydriasis	0.00	12.00	2.00	5.50
			Prostration	0.08	12.00	1.92	5.92
			Death	-	-	-	-
40	H237	08/23/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	240.00	1.75	5.75
			Convulsions	1.68	1.75	0.07	0.07
			Salivation	0.00	2.50	2.00	2.50
			Miosis	4.50	48.00	0.00	1.50
			Mydriasis	0.00	0.25	0.25	0.25
			Prostration	0.03	48.00	1.97	2.97
			Death	-	-	-	-
40	6XM	08/24/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	6.00	2.00	4.50
			Convulsions	-	-	0.00	0.00
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	96.00	2.00	6.00
			Prostration	0.55	72.00	1.45	5.45
			Death	-	-	-	-
39	7BK	08/24/93	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	43.83	2.00	6.00
			Convulsions	0.40	4.00	0.60	2.10
			Salivation	1.75	12.00	0.25	4.25
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	43.83	2.00	6.00
			Prostration	0.08	43.83	1.92	5.92
			Death	43.83	-	-	-

[a] Based on weight losses of syringes and concentration analysis of dosing solution.
[b] Sign was not noted during duration of the experiment.
[c] Excessive salivation/bronchial secretions.
[d] Euthanatized on day 3 because of moribund condition.

AMENDED TABLE D-20. STATISTICAL COMPARISONS BETWEEN CLINICAL SIGNS FOR PHASE III AND PHASE IV EXPERIMENTS

Clinical Sign	Incidence of Clinical Signs			Fisher's Exact Test p-values		Endpoint	Wilcoxon Test p-values		Analyses of Variance Results Using Censored Values							
									Group 1 Predicted		Group 2 Predicted		Group 3 Predicted		Chi-Square p-value	
	Group 1	Group 2	Group 3	Grps 1&3	Grps 2&3		Grps 1&3	Grps 2&3	Mean (hr)	(S.E.)	Mean (hr)	(S.E.)	Mean (hr)	(S.E.)	Grps 1&3	Grps 2&3
Tremors	4/4	30/30	10/10	1.000	1.000	Time to onset	1.000	1.000	0.00	(0.00)	0.00	(0.00)	0.00	(0.00)	1.000	1.000
						Duration, 1st 2 hrs	0.100	0.089	0.88	(0.23)	1.58	(0.09)	1.86	(0.15)	0.000[a]	0.086
						Duration, 1st 6 hrs	0.193	0.149	1.88	(0.78)	4.18	(0.30)	4.92	(0.52)	0.004[a]	0.182
Convulsions	2/4	22/30	7/10	0.580	1.000	Time to onset	0.556	0.192	6.90	(15.9)	1.63	(1.28)	2.94	(4.07)	0.757	0.710
						Duration, 1st 2 hrs	0.563	0.911	0.30	(0.27)	0.44	(0.10)	0.44	(0.16)	0.603	0.995
						Duration, 1st 6 hrs	0.563	0.899	0.35	(0.40)	0.62	(0.14)	0.79	(0.24)	0.472	0.535
Salivation/ Bronchial Secretions	4/4	27/30	8/10	1.000	0.584	Time to onset	0.181	0.746	0.01	(0.02)	0.16	(0.10)	0.18	(0.19)	0.108	0.912
						Duration, 1st 2 hrs	1.000	0.404	1.55	(0.42)	1.00	(0.13)	1.20	(0.22)	0.469	0.438
						Duration, 1st 6 hrs	0.570	0.572	5.43	(1.89)	2.72	(0.42)	3.20	(0.73)	0.276	0.579
Miosis	2/4	23/30	6/10	1.000	0.418	Time to onset	0.129	0.586	0.53	(0.77)	8.46	(4.35)	12.80	(11.7)	0.215	0.691
						Duration, 1st 2 hrs	0.288	0.789	0.56	(0.23)	0.20	(0.09)	0.20	(0.15)	0.238	1.000
						Duration, 1st 6 hrs	0.754	0.795	1.56	(0.96)	1.35	(0.35)	1.15	(0.61)	0.709	0.769
Mydriasis	4/4	30/30	10/10	1.000	1.000	Time to onset	1.000	1.000	0.00	(0.00)	0.00	(0.00)	0.00	(0.00)	1.000	1.000
						Duration, 1st 2 hrs	0.421	0.974	0.86	(0.38)	1.36	(0.13)	1.39	(0.22)	0.316	0.893
						Duration, 1st 6 hrs	0.430	0.802	1.59	(1.22)	2.70	(0.41)	3.47	(0.70)	0.247	0.350
Prostration	4/4	30/30	10/10	1.000	1.000	Time to onset	0.136	0.179	0.04	(0.01)	0.05	(0.00)	0.07	(0.01)	0.204	0.055
						Duration, 1st 2 hrs	0.943	0.682	1.47	(0.22)	1.70	(0.07)	1.83	(0.13)	0.144	0.340
						Duration, 1st 6 hrs	0.395	0.950	3.79	(1.24)	4.21	(0.39)	4.78	(0.68)	0.391	0.452
Death [b]	4/4	6/30	2/10	0.015[a]	1.000	Time to death	1.000	0.739	2.41	(4.44)	866	(1,116)	793	(1438)	0.019[a]	0.969

Explanation of column headings

Incidence of Clinical Sign: Proportion of animals in each group that exhibited the clinical sign.

Group definitions are: Group 1 = Phase III Control group
Group 2 = Phase III pooled 4-24 μg/kg i.m. PYR groups
Group 3 = Phase IV 40 μg/kg i.g. PYR group

Fisher's Exact Test: p-value for comparing the incidence of clinical signs between pairs of study groups.

Wilcoxon Test: p-value for Wilcoxon's rank sum test comparing nonmissing, uncensored durations between pairs of study groups.

Analyses of Variance Using Censored Values:

Predicted Mean: The mean value of endpoint predicted by the ANOVA for each group. S.E. is the standard error of the predicted mean.

Chi-square p-value: p-value for comparing the mean predicted values between pairs of groups.

[a] Statistically significant at the 0.05 level.

[b] Analysis of time to death based on 48 hr endpoint.

AMENDED TABLE D-21. DATA LISTING OF CLINICAL SIGN ENDPOINTS FROM PHASE V

Treatment Group	Calculated GD Dose[a] (µg/kg)	Animal	Clinical Sign	Time to onset (hr)	Time to last obs (hr)	Duration of sign in 1st 2 hr (hrs)	Duration of sign in 1st 6 hr (hrs)
ATR/2-PAM	5.7	H482	Appears Normal	-[b]	-	0.00	0.00
			Tremors	-	-	0.00	0.00
			Convulsions	-	-	0.00	0.00
			Salivation[c]	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	96.00	2.00	6.00
			Prostration	-	-	0.00	0.00
			Death	-	-	-	-
ATR/2-PAM	6.9	66P	Appears Normal	192.00	240.00	0.00	0.00
			Tremors	0.00	192.00	2.00	5.50
			Convulsions	-	-	0.00	0.00
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	96.00	2.00	6.00
			Prostration	-	-	0.00	0.00
			Death	-	-	-	-
ATR/2-PAM	8.5	75P	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	4.50	2.00	4.50
			Convulsions	-	-	0.00	0.00
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	168.00	2.00	6.00
			Prostration	0.18	1.25	1.07	1.07
			Death	-	-	-	-
ATR/2-PAM	7.9	7AC	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	0.72	0.72	0.72
			Convulsions	-	-	0.00	0.00
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	0.72	0.72	0.72
			Prostration	0.13	0.72	0.58	0.58
			Death	0.72	-	-	-
ATR/2-PAM	8.5	74A	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	3.50	2.00	3.50
			Convulsions	-	-	0.00	0.00
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.25	240.00	1.75	5.75
			Prostration	-	-	0.00	0.00
			Death	-	-	-	-
ATR/2-PAM	10.0	6VZ	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	0.83	0.83	0.83
			Convulsions	0.20	0.75	0.55	0.55
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	0.83	0.83	0.83
			Prostration	0.20	0.83	0.63	0.63
			Death	0.83	-	-	-

AMENDED TABLE D-21.
(Continued)

Treatment Group	Calculated GD Dose[a] (µg/kg)	Animal	Clinical Sign	Time to onset (hr)	Time to last obs. (hr)	Duration of sign in 1st 2 hr (hrs)	Duration of sign in 1st 6 hr (hrs)
ATR/2-PAM	13.2	H258	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	0.75	0.75	0.75
			Convulsions	0.10	0.50	0.40	0.40
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	0.75	0.50	0.50
			Prostration	0.12	0.75	0.63	0.63
			Death	0.75	-	-	-
ATR/2-PAM	13.3	6RB	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	1.33	1.33	1.33
			Convulsions	-	-	0.00	0.00
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	1.25	1.25	1.25
			Prostration	0.12	1.33	1.22	1.22
			Death	1.33	-	-	-
ATR/2-PAM/DZM	5.8	7CK	Appears Normal	168.00	240.00	0.00	0.00
			Tremors	0.00	0.75	0.75	0.75
			Convulsions	-	-	0.00	0.00
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	168.00	2.00	6.00
			Prostration	-	-	0.00	0.00
			Death	-	-	-	-
ATR/2-PAM/DZM	6.6	71G	Appears Normal	168.00	240.00	0.00	0.00
			Tremors	0.75	1.75	0.75	0.75
			Convulsions	-	-	0.00	0.00
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	96.00	2.00	6.00
			Prostration	-	-	0.00	0.00
			Death	-	-	-	-
ATR/2-PAM/DZM	7.3	78V	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	12.00	2.00	6.00
			Convulsions	-	-	0.00	0.00
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	240.00	2.00	6.00
			Prostration	0.15	0.50	0.35	0.35
			Death	-	-	-	-
ATR/2-PAM/DZM	10.9	6S4	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	1.25	1.25	1.25
			Convulsions	0.12	0.50	0.38	0.38
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	1.27	1.27	1.27
			Prostration	0.12	1.27	1.15	1.15
			Death	1.27	-	-	-

AMENDED TABLE D-21.
(Continued)

Treatment Group	Calculated GD Dose[a] (µg/kg)	Animal	Clinical Sign	Time to onset (hr)	Time to last obs. (hr)	Duration of sign in 1st 2 hr (hrs)	Duration of sign in 1st 6 hr (hrs)
ATR/2-PAM/DZM	11.6	75U	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	0.75	0.75	0.75
			Convulsions	-	-	0.00	0.00
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	0.83	0.83	0.83
			Prostration	0.10	0.83	0.73	0.73
			Death	0.83	-	-	-
ATR/2-PAM/DZM	12.3	7AU	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	0.70	0.45	0.45
			Convulsions	0.10	0.50	0.40	0.40
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	0.70	0.70	0.70
			Prostration	0.07	0.70	0.63	0.63
			Death	0.70	-	-	-
ATR/2-PAM/DZM	15.3	6W6	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	0.42	0.42	0.42
			Convulsions	-	-	0.00	0.00
			Salivation	0.25	0.42	0.17	0.17
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	0.42	0.42	0.42
			Prostration	0.08	0.42	0.33	0.33
			Death	0.42	-	-	-
ATR/2-PAM/DZM	17.9	7CC	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	12.00	2.00	6.00
			Convulsions	-	-	0.00	0.00
			Salivation	6.00	70.67	0.00	0.00
			Miosis	18.00	70.67	0.00	0.00
			Mydriasis	0.00	12.00	2.00	6.00
			Prostration	0.07	70.67	1.93	5.93
			Death	70.67	-	-	-
ATR/2-PAM/DZM	19.4	H453	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	2.50	2.00	2.50
			Convulsions	-	-	0.00	0.00
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	2.83	2.00	2.83
			Prostration	0.03	2.83	1.97	2.80
			Death	2.83	-	-	-
ATR/2-PAM/DZM	28.5	H264	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	1.25	1.25	1.25
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	0.50	0.50	0.50
			Miosis	1.00	1.50	0.50	0.50
			Mydriasis	0.00	1.00	1.00	1.00
			Prostration	0.03	1.50	1.47	1.47
			Death	1.50	-	-	-

AMENDED TABLE D-21.
(Continued)

Treatment Group	Calculated GD Dose[a] (µg/kg)	Animal	Clinical Sign	Time to onset (hr)	Time to last obs. (hr)	Duration of sign in 1st 2 hr (hrs)	Duration of sign in 1st 6 hr (hrs)
PYR/ATR/2-PAM	79.4	H398	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	12.00	2.00	6.00
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	24.00	0.75	1.75
			Miosis	0.00	48.00	0.25	3.75
			Mydriasis	0.25	2.50	1.75	2.25
			Prostration	0.02	72.00	1.98	5.98
			Death[d]	-	-	-	-
PYR/ATR/2-PAM	129.4	6WG	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	5.00	2.00	4.00
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	48.00	1.00	3.00
			Miosis	0.00	96.00	2.00	6.00
			Mydriasis	0.00	0.25	0.25	0.25
			Prostration	0.05	96.00	1.95	5.95
			Death[e]	-	-	-	-
PYR/ATR/2-PAM	158.3	6TY	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	96.00	2.00	6.00
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	12.00	1.00	2.50
			Miosis	0.00	12.00	1.25	5.25
			Mydriasis	0.00	1.50	1.00	1.00
			Prostration	0.02	144.00	1.98	5.98
			Death[f]	-	-	-	-
PYR/ATR/2-PAM	173.8	73C	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	12.00	1.75	5.75
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	24.00	0.75	2.25
			Miosis	0.00	72.00	2.00	6.00
			Mydriasis	0.00	0.25	0.25	0.25
			Prostration	0.03	72.00	1.97	5.97
			Death[d]	-	-	-	-
PYR/ATR/2-PAM	209.4	75Z	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	12.00	2.00	6.00
			Convulsions	0.35	0.75	0.40	0.40
			Salivation	0.00	72.00	2.00	5.00
			Miosis	0.00	48.00	2.00	6.00
			Mydriasis	0.00	0.25	0.25	0.25
			Prostration	0.03	72.00	1.97	5.97
			Death[d]	-	-	-	-
PYR/ATR/2-PAM	212.3	H525	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	0.08	0.08	0.08
			Convulsions	-	-	0.00	0.00
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	0.08	0.08	0.08
			Prostration	0.05	0.08	0.03	0.03
			Death	0.08	-	-	-

AMENDED TABLE D-21.
(Continued)

Treatment Group	Calculated GD Dose[a] (µg/kg)	Animal	Clinical Sign	Time to onset (hr)	Time to last obs. (hr)	Duration of sign in 1st 2 hr (hrs)	Duration of sign in 1st 6 hr (hrs)
PYR/ATR/2-PAM	199.7	6T4	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	0.08	0.08	0.08
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	0.08	0.08	0.08
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	0.08	0.08	0.08
			Prostration	0.03	0.08	0.05	0.05
			Death	0.08	-	-	-
PYR/ATR/2-PAM	208.3	73P	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	0.67	0.67	0.67
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	0.50	0.50	0.50
			Miosis	0.00	0.67	0.67	0.67
			Mydriasis	0.00	0.25	0.25	0.25
			Prostration	0.03	0.67	0.63	0.63
			Death	0.67	-	-	-
PYR/ATR/2-PAM	208.4	H074	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	2.50	2.00	2.50
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	47.92	1.50	5.50
			Miosis	0.00	12.00	1.00	5.00
			Mydriasis	0.00	0.75	0.75	0.75
			Prostration	0.03	47.92	1.97	5.97
			Death	47.92	-	-	-
PYR/ATR/2-PAM	257.8	H585	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	1.00	1.00	1.00
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	30.58	0.25	4.25
			Miosis	0.00	30.58	2.00	6.00
			Mydriasis	0.00	0.50	0.50	0.50
			Prostration	0.03	30.58	1.97	5.97
			Death	30.58	-	-	-
PYR/ATR/2-PAM/DZM	60.3	79P	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	12.00	0.25	0.75
			Convulsions	0.13	0.25	0.12	0.12
			Salivation	2.50	4.00	0.00	1.50
			Miosis	0.25	240.00	1.25	5.25
			Mydriasis	0.00	0.25	0.25	0.25
			Prostration	0.03	12.00	1.97	5.97
			Death	-	-	-	-
PYR/ATR/2-PAM/DZM	76.1	H472	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	0.73	0.73	0.73
			Convulsions	-	-	0.00	0.00
			Salivation	-	-	0.00	0.00
			Miosis	0.00	0.73	0.73	0.73
			Mydriasis	0.00	0.25	0.25	0.25
			Prostration	0.03	0.73	0.70	0.70
			Death	0.73	-	-	-

AMENDED TABLE D-21.
(Continued)

Treatment Group	Calculated GD Dose[a] (µg/kg)	Animal	Clinical Sign	Time to onset (hr)	Time to last obs. (hr)	Duration of sign in 1st 2 hr (hrs)	Duration of sign in 1st 6 hr (hrs)
PYR/ATR/2-PAM/DZM	75.4	7C6	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	12.00	2.00	6.00
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	72.00	1.00	1.00
			Miosis	0.25	72.00	1.75	5.75
			Mydriasis	0.00	0.25	0.25	0.25
			Prostration	0.03	72.00	1.97	4.47
			Death[d]	-	-	-	-
PYR/ATR/2-PAM/DZM	78.1	5R2	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	164.58	2.00	6.00
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	72.00	1.50	2.50
			Miosis	3.00	48.00	0.00	3.00
			Mydriasis	0.00	0.25	0.25	0.25
			Prostration	0.03	164.58	1.97	5.97
			Death	164.58	-	-	-
PYR/ATR/2-PAM/DZM	96.4	7C4	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	47.67	2.00	6.00
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	47.67	1.75	2.75
			Miosis	0.00	47.67	0.75	4.25
			Mydriasis	0.00	0.25	0.25	0.25
			Prostration	0.03	47.67	1.97	5.97
			Death	47.67	-	-	-
PYR/ATR/2-PAM/DZM	108.8	H309	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	0.73	0.73	0.73
			Convulsions	0.28	0.50	0.22	0.22
			Salivation	0.00	0.73	0.73	0.73
			Miosis	0.00	0.73	0.73	0.73
			Mydriasis	0.00	0.25	0.25	0.25
			Prostration	0.02	0.73	0.72	0.72
			Death	0.73	-	-	-
PYR/ATR/2-PAM/DZM	127.9	H300	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	0.75	0.75	0.75
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	0.25	0.25	0.25
			Miosis	0.00	1.73	1.73	1.73
			Mydriasis	0.00	0.25	0.25	0.25
			Prostration	0.03	1.73	1.70	1.70
			Death	1.73	-	-	-
PYR/ATR/2-PAM/DZM	158.6	7D6	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	144.00	2.00	6.00
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	96.00	1.00	2.00
			Miosis	0.00	72.00	2.00	6.00
			Mydriasis	0.00	0.25	0.25	0.25
			Prostration	0.02	144.00	1.98	5.98
			Death[f]	-	-	-	-

AMENDED TABLE D-21.
(Continued)

Treatment Group	Calculated GD Dose[a] (µg/kg)	Animal	Clinical Sign	Time to onset (hr)	Time to last obs. (hr)	Duration of sign in 1st 2 hr (hrs)	Duration of sign in 1st 6 hr (hrs)
PYR/ATR/2-PAM/DZM	198.7	H612	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	0.75	0.75	0.75
			Convulsions	0.18	0.75	0.57	0.57
			Salivation	0.00	0.75	0.75	0.75
			Miosis	0.00	0.50	0.50	0.50
			Mydriasis	0.00	0.75	0.50	0.50
			Prostration	0.03	0.75	0.72	0.72
			Death	0.75	-	-	-
PYR/ATR/2-PAM/DZM	258.9	7AL	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	5.50	2.00	3.00
			Convulsions	-	-	0.00	0.00
			Salivation	0.00	12.00	2.00	5.50
			Miosis	0.00	12.00	2.00	6.00
			Mydriasis	0.00	0.25	0.25	0.25
			Prostration	0.03	12.00	1.97	5.97
			Death	20.75	-	-	-
ATR/2-PAM (Cage)	20.1	5WT	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	1.25	1.25	1.25
			Convulsions	-	-	0.00	0.00
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	1.47	1.47	1.47
			Prostration	0.07	1.47	1.40	1.40
			Death	1.47	-	-	-
ATR/2-PAM (Cage)	20.6	I438	Appears Normal	-	-	0.00	0.00
			Tremors	0.00	24.00	2.00	5.50
			Convulsions	-	-	0.00	0.00
			Salivation	0.25	27.68	0.25	0.75
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	27.68	2.00	6.00
			Prostration	0.12	27.68	1.88	5.88
			Death	27.68	-	-	-
ATR/2-PAM (Cage)	20.7	6W8	AppearsNormal	-	-	0.00	0.00
			Tremors	0.00	48.00	2.00	5.00
			Convulsions	-	-	0.00	0.00
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	72.00	2.00	6.00
			Prostration	0.17	72.00	1.83	5.33
			Death[d]	-	-	-	-
ATR/2-PAM (Cage)	20.6	74H	AppearsNormal	-	-	0.00	0.00
			Tremors	0.00	0.50	0.50	0.50
			Convulsions	-	-	0.00	0.00
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	0.75	0.75	0.75
			Prostration	0.10	0.75	0.65	0.65
			Death	0.75	-	-	-

AMENDED TABLE D-21.
(Continued)

Treatment Group	Calculated GD Dose[a] (µg/kg)	Animal	Clinical Sign	Time to onset (hr)	Time to last obs. (hr)	Duration of sign in 1st 2 hr (hrs)	Duration of sign in 1st 6 hr (hrs)
ATR/2-PAM (Cage)	20.6	7C9	AppearsNormal	-	-	0.00	0.00
			Tremors	0.00	1.72	1.72	1.72
			Convulsions	-	-	0.00	0.00
			Salivation	-	-	0.00	0.00
			Miosis	-	-	0.00	0.00
			Mydriasis	0.00	1.72	1.72	1.72
			Prostration	0.08	1.72	1.63	1.63
			Death	1.72	-	-	-

[a] GD doses calculated from weight losses of syringes and chemical analysis of dosing solution.
[b] Sign was not noted during duration of the experiment.
[c] Excessive salivation or bronchial secretions.
[d] Euthanatized on day 3 because of moribund condition.
[e] Euthanatized on day 4 because of moribund condition.
[f] Euthanatized on day 6 because of moribund condition.

AMENDED TABLE D-23. SUMMARY OF STATISTICAL COMPARISONS BETWEEN CLINICAL SIGNS FOR FOUR TREATMENT GROUPS FROM PHASE V EXPERIMENTS

Clinical Sign	Incidence of Clinical Sign				Fisher's Exact Test	Endpoint	Wilcoxon Test p-value	Analysis of Variance Results Using Censored Values								Chi-Square p-value	Log-Dose Slope p-value
								2-PAM Predicted		2-PAM/DZM Predicted		PYR/2-PAM Predicted		PYR/2-PAM/DZM Predicted			
	ATR/ 2-PAM	ATR/ 2-PAM/DZM	PYR/ATR/ 2-PAM	PYR/ATR/ 2-PAM/DZM				Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)		
Tremors	7/8	10/10	10/10	10/10	0.474	Time, 1st onset	-	0.04	(0.02)	0.02	(0.01)	0.01	(0.00)	0.01	(0.01)	0.460	0.072
						Duration, 2 hrs	0.605	1.64	(0.26)	1.36	(0.20)	1.83	(0.22)	1.61	(0.21)	0.478	-
						Duration, 6 hrs	0.308	3.72	(0.91)	2.55	(0.69)	4.51	(0.75)	4.16	(0.74)	0.228	-
Convulsions	2/8	2/10	1/10	3/10	1.000	Time, 1st onset	-	NE		NE		NE		NE		0.586	0.321
						Duration, 2 hrs	0.806	0.12	(0.06)	0.08	(0.05)	0.04	(0.05)	0.10	(0.05)	0.789	-
						Duration, 6 hrs	0.806	0.12	(0.06)	0.08	(0.05)	0.04	(0.05)	0.10	(0.05)	0.789	-
Salivation/ Bronchial Secretions	0/8	3/10	9/10	9/10	0.000[a]	Time, 1st onset	-	NE		27.1	(23.6)	0.01	(0.01)	0.06	(0.04)	0.686	0.000[a]
						Duration, 2 hrs	0.000[a]	0.00	(0.17)	0.10	(0.15)	0.87	(0.15)	0.99	(0.16)	0.000[a]	-
						Duration, 6 hrs	0.000[a]	0.00	(0.43)	0.16	(0.39)	2.75	(0.40)	2.02	(0.41)	0.000[a]	-
Miosis	0/8	2/10	8/10	10/10	0.000[a]	Time, 1st onset	-	NE		115	(95.7)	0.02	(0.01)	0.05	(0.03)	0.534	0.000[a]
						Duration, 2 hrs	0.000[a]	0.00	(0.20)	0.08	(0.18)	1.19	(0.19)	1.33	(0.20)	0.000[a]	-
						Duration, 6 hrs	0.000[a]	0.00	(0.53)	0.16	(0.48)	4.23	(0.50)	4.42	(0.56)	0.000[a]	-
Mydriasis	8/8	10/10	10/10	10/10	1.000	Time, 1st onset	-	0.02	(0.00)	0.01	(0.00)	0.01	(0.00)	0.01	(0.00)	0.159	0.100
						Duration, 2 hrs	0.000[a]	1.80	(0.15)	1.84	(0.14)	0.63	(0.12)	0.30	(0.11)	0.000[a]	-
						Duration, 6 hrs	0.000[a]	5.00	(0.55)	5.02	(0.54)	0.83	(0.41)	0.37	(0.39)	0.000[a]	-
Prostration	5/8	8/10	10/10	10/10	0.017[a]	Time, 1st onset	-	3.60	(3.09)	0.58	(0.44)	0.03	(0.02)	0.05	(0.04)	0.108	0.001[a]
						Duration, 2 hrs	0.001[a]	0.73	(0.21)	1.15	(0.20)	1.97	(0.20)	2.02	(0.21)	0.000[a]	-
						Duration, 6 hrs	0.000[a]	1.06	(0.49)	2.27	(0.49)	5.97	(0.46)	5.72	(0.49)	0.000[a]	-
Death[b]	4/8	6/10	5/10	6/10	1.000	Time to death	-	25.1	(31.8)	22.6	(26.3)	29.6	(34.0)	62.3	(74.0)	0.030[a]	0.003[a]

Explanation of column headings

Incidence of clinical sign: proportion of animals in each group that exhibited the clinical sign.

Fisher's Exact Test: p-value for test comparing the incidence of clinical signs between non-PYR pretreated animals (ATR/2-PAM and ATR/2-PAM/DZM) and PYR pretreated animals (PYR/ATR/2-PAM and PYR/ATR/2-PAM/DZM).

Wilcoxon Test: p-value for Wilcoxon's rank sum test comparing nonmissing, uncensored durations between non-PYR pretreated animals (ATR/2-PAM and ATR/2-PAM/DZM) and PYR pretreated animals (PYR/ATR/2-PAM and PYR/ATR/2-PAM/DZM). This test was not performed on times to onset since they were determined to be dose-dependent.

Analyses of Variance Using Censored Values:

Predicted Mean: The mean value of endpoint predicted by the ANOVA for each group. S.E. is the standard error of the predicted mean. Predicted values were computed at the GD 48 hr LD_{50} for each group, 8.81 µg/kg for ATR/2-PAM, 11.1 µg/kg for ATR/2-PAM/DZM, 182 µg/kg for PYR/ATR/2-PAM, and 94.5 µg/kg for PYR/ATR/2-PAM/DZM.

Chi-square p-value: p-value for comparing the mean prediced values among all four treatment groups.

Log-dose Slope p-value: p-value for the log GD dose slope. P-values for the log-dose slope were not significant for durations within 2 hr and 6 hr, so the log GD dose covariate was dropped from the model for duration.

NE - Mean time to onset was not estimable due to the large number of censored values.

[a] Statistically significant at the 0.05 level.

[b] Analysis of time to death based on 48 hr endpoint.

Table D-23 summarizes the results of the statistical analysis for the Phase V clinical signs data, and are comparable to those in Tables D-14, D-17, and D-20. Clinical signs information from the 5 animals injected with GD while restrained within their cages and treated with ATR/2-PAM were not included in the statistical analysis. Comparisons of the incidence of clinical signs between PYR pretreated monkeys and animals not pretreated were made using Fisher's Exact Test. Similarly, durations of clinical signs were compared between these two groups of animals using Wilcoxon's Test. Times to onset and durations were analyzed by oneway ANOVAs; a covariate for log GD dose was included in the ANOVAs for times to onset. Because ANOVA models for times to onset included a covariate for log GD dose, mean predicted times to onset were computed at the 48-hr GD LD_{50} for each group: 8.8 μg/kg, 11.1 μg/kg, 182 μg/kg, and 94.5 μg/kg for ATR/2-PAM, ATR/2-PAM/DZM, PYR/ATR/2-PAM, and PYR/ATR/2-PAM/DZM treated animals, respectively.

Conclusions derived from the analyses of the Phase V clinical signs data, at the 5 percent significance level, are:

1) Mean predicted incidence and duration of salivation, miosis, and prostration for PYR pretreated animals were statistically greater than those for animals not given a PYR pretreatment.

2) Mean predicted duration of mydriasis for PYR pretreated animals was statistically less than that for animals not given a PYR pretreatment.

3) Log GD dose was significantly related to times to onset of salivation, miosis, and prostration, and time to death, with times to onset predicted to decrease with increasing GD dose.

4) Mean predicted time to death for PYR pretreated animals was significantly greater than that for animals not given a PYR pretreatment.

for times to onset. Mean times to onset and durations, based on the ANOVAs, were predicted for each clinical sign. Because ANOVA models for times to onset included a covariate for log GD dose, mean predicted times to onset were computed at the 48-hr GD LD_{50} for each group: 20.5 and 6.5 μg/kg for ATR/2-PAM treated and untreated animals, respectively. A chi-square test was conducted to compare the mean predicted endpoints between ATR/2-PAM treated and untreated animals for each clinical sign. The chi-square p-values are displayed in column nine of the table.

Conclusions derived from the analysis of the Phase I clinical signs data, at the 5 percent significance level, are:

1) There are no statistical differences between incidence of clinical signs for ATR/2-PAM treated and untreated animals.

2) Log GD dose was significantly related to times to onset of tremors, convulsions, salivation, miosis, and prostration, and time to death, with times to onset predicted to decrease with increasing GD dose.

3) Mean predicted times to onset of convulsions, salivation, and miosis for ATR/2-PAM treated animals were significantly greater than those for untreated animals.

4) Mean predicted duration of convulsions for ATR/2-PAM treated animals was significantly less than that for untreated animals.

5) Mean predicted duration of mydriasis within the first 2 hr for ATR/2-PAM treated animals was significantly greater than that for untreated animals.

3.1.6.2 Phase III

A listing of clinical signs data for Phase III animals is presented in Table D-15 in Appendix D. Table D-16 in Appendix D displays simple descriptive statistics for the PYR pretreated and untreated animals, using uncensored, nonmissing values.